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EVOLUTIONARY ADAPTATION TO TEMPERATURE. VI. PHENOTYPIC ACCLIMATION AND ITS EVOLUTION IN *ESCHERICHIA COLI*

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Abstract.—Acclimation refers to reversible, nongenetic changes in phenotype that are induced by specific environmental conditions. Acclimation is generally assumed to improve function in the environment that induces it (the beneficial acclimation hypothesis). In this study, we experimentally tested this assumption by measuring relative fitness of the bacterium *Escherichia coli* acclimated to different thermal environments. The beneficial acclimation hypothesis predicts that bacteria acclimated to the temperature of competition should have greater fitness than do bacteria acclimated to any other temperature. The benefit predicted by the hypothesis was found in only seven of 12 comparisons; in the other comparisons, either no statistically demonstrable benefit was observed or a detrimental effect of acclimation was demonstrated. For example, in a lineage evolutionarily adapted to 37°C, bacteria acclimated to 37°C have a higher fitness at 32°C than do bacteria acclimated to 32°C, a result exactly contrary to prediction; acclimation to 27°C or 40°C prior to competition at those temperatures confers no benefit over 37°C acclimated forms. Consequently, the beneficial acclimation hypothesis must be rejected as a general prediction of the inevitable result of phenotypic adjustments associated with new environments. However, the hypothesis is supported in many instances when the acclimation and competition temperatures coincide with the historical temperature at which the bacterial populations have evolved. For example, when the evolutionary temperature of the population was 37°C, bacteria acclimated to 37°C had superior fitness at 37°C to those acclimated to 32°C; similarly, bacteria evolutionarily adapted to 32°C had a higher fitness during competition at 32°C than they did when acclimated to 37°C. The more surprising results are that when the bacteria are acclimated to their historical evolutionary temperature, they are frequently competitively superior even at other temperatures. For example, bacteria that have evolved at either 20°C or 32°C and are acclimated to their respective evolutionary temperatures have a greater fitness at 37°C than when they are acclimated to 37°C. Thus, acclimation to evolutionary temperature may, as a correlated consequence, enhance performance not only in the evolutionary environment, but also in a variety of other thermal environments.

Key words.—Acclimation, adaptation, bacteria, beneficial acclimation hypothesis, *Escherichia coli*, fitness, phenotypic plasticity, temperature.

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The exposure of organisms to new environments often causes reversible, nongenetic changes in the expression of a variety of phenotypic traits. This general response is termed phenotypic plasticity, and its specific form in different thermal environments is called acclimation (Rome et al. 1992; Scheiner 1993; Huey and Berrigan 1996). These acclimation responses may involve changes in physiological rate processes and performance, thermal niche limits, behaviors, and even morphological characters (e.g., Prosser 1973; Hochachka and Somero 1984; Cossins and Bowler 1987). These alterations in phenotype have generally been assumed to benefit the performance of the organism in its new thermal environment and to confer an advantage that would otherwise be lacking without acclimation (e.g., Levins 1969; Hochachka and Somero 1984; Hoffmann and Parsons 1991; Rome et al. 1992). Recently, this assumption, which we call the beneficial acclimation hypothesis, has attracted more general discussion and specific experimental evaluation (Leroi et al. 1994a; Hoffmann 1995; Padilla and Adolph 1996; Huey and Berrigan 1996). Experimental studies examining acclimation and subsequent performance in different thermal environments (Krebs and Loeschcke 1994; Leroi et al. 1994a; Zamudio et al. 1995; Huey and Berrigan 1996 reanalysis of data from Zwaan et al. 1992) have all found instances in which organisms have inferior performance at their acclimation temperatures, calling into serious question the generality of the beneficial acclimation hypothesis. Studies of phenotypic adjustment to different light regimes have produced mixed results, some supporting (Kingsolver 1995; Schmitt et al. 1995; Dudley and Schmitt 1996) and others falsifying (Rice and Bazzaz 1989) the hypothesis that such adjustments are necessarily beneficial.

The research presented here is an outgrowth of our earlier study (Leroi et al. 1994a) examining the effect of acclimation temperature on relative fitness in the bacterium *Escherichia coli*. In that study, we acclimated genetically marked (but otherwise isogenic) clonal populations of this bacterium to 32°C and to 41.5°C. After acclimation, these marked clones were reciprocally competed at 32°C and 41.5°C. As predicted by the beneficial acclimation hypothesis, at 32°C the population acclimated to 32°C had a greater fitness than that acclimated to 41.5°C. However, at 41.5°C, the fitness of the 32°C-acclimated population was again greater, contrary to the predictions of the hypothesis. The generality of the hypothesis was therefore falsified. In this study, we examine how specific these results were to this particular pairing of environmental temperatures. The bacterium utilized in the previous study had evolved at 37°C (Lenski et al. 1991) and exposure to 41.5°C is clearly stressful to it (Lenski and Bennett 1993), being less than 1°C from its upper lethal temperature (Bennett and Lenski 1993). Was the failure to find acclimation benefit related to the stressful nature of the environment? How general is beneficial acclimation in other, nonstressful portions of this organism's thermal niche? Can

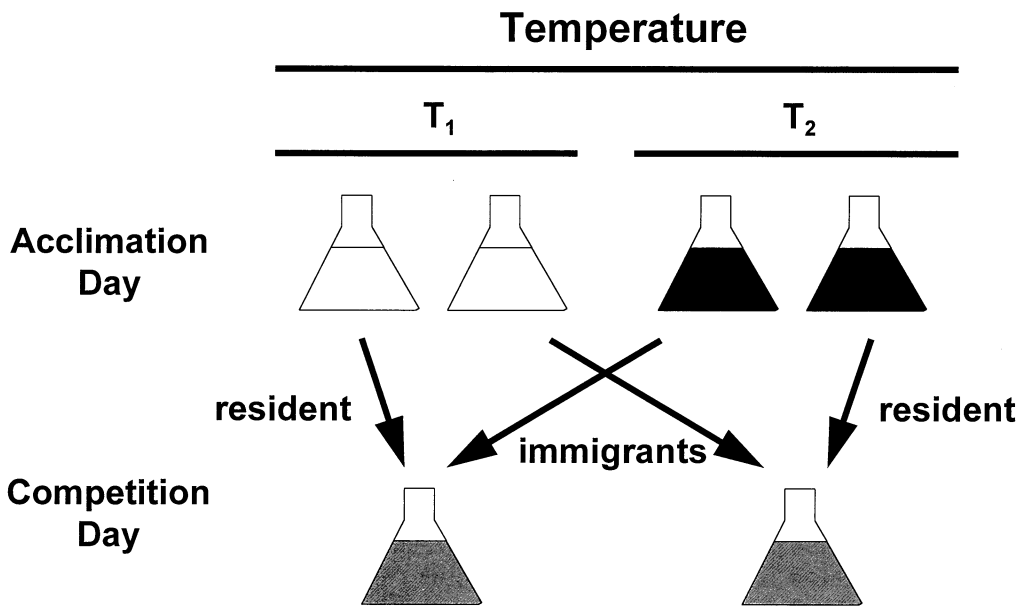

FIG. 1. Experimental design to investigate the effects of acclimation on fitness in the ancestral bacterium. Genetically marked (but otherwise isogenic) clones are grown separately at each of two different temperatures (T_1 and T_2) on the acclimation day. They are then cross-competed on the competition day, the resident competitor remaining at its acclimation temperature and the immigrant moving to the alternative temperature. The fitness of the resident relative to the immigrant is measured over that first day of competition.

the patterns of absence or presence of acclimation benefit be understood in reference to the evolutionary history of adaptation to different thermal environments? This study investigates each of these questions.

MATERIALS AND METHODS

Study Organisms

The bacteria used in this study were all derived from a single genotype (REL1206) of *E. coli* B, which was isolated from a population that had been maintained for 2000 generations at 37°C by serial transfer in minimal glucose medium (Lenski et al. 1991). This genotype cannot metabolize arabinose (Ara^-). An arabinose-utilizing mutant genotype (Ara^+) was derived from Ara^-, the genotypes being otherwise identical. This trait is used as a marker in competition experiments to estimate relative fitness; the marker's selective neutrality has been previously verified under several thermal regimes (Bennett et al. 1992) and is reaffirmed in the present study. This pair of genotypes was utilized in the studies on phenotypic acclimation described in the next section. They were also the ancestral genotypes used to found an evolutionary experiment on temperature adaptation (previously described in Bennett et al. 1992 and Mongold et al. 1996). To examine the effect of evolutionary adaptation on phenotypic acclimation responses described in the subsequent section, we used genotypes that had evolved at 32°C and at 20°C in these previous studies. The 32°C and 20°C groups each consisted of six independently derived populations (lines) that were maintained by serial transfer at their respective temperatures for 2000 generations. A single genotype randomly chosen from each population was used in the analysis of evolutionary changes in the effects of phenotypic acclimation on fitness.

Fitness Effects of Phenotypic Acclimation in the Ancestor

The Ara^- and Ara^+ genotypes were used to investigate the effects on competitive fitness of reciprocal acclimation to several pairs of different thermal environments. These experiments follow the protocol described in Leroi et al. (1994a). Briefly, reciprocally marked clones (Ara^- and Ara^+) are acclimated to two different temperatures for one day (6–7 generations) and then are allowed to cross-compete on the next day at both their own acclimation temperature and that of the other acclimation state (see Fig. 1 for a schematic diagram of the experiment). In each competition environment, the clone that was acclimated to the competition temperature is termed the "resident" and the clone acclimated to the other temperature is designated the "immigrant." Relative fitness of the two acclimation states is measured by their differential rates of offspring production, using the following equation:

$$W = \log (R_f/R_i)/\log (I_f/I_i), \quad (1)$$

where subscripts i and f denote initial and final values for the population densities of the resident (R) and immigrant (I) acclimation states. Note that fitness in these experiments is always expressed as resident relative to immigrant. $W = 1$ implies no fitness advantage to either acclimation state. The beneficial acclimation hypothesis predicts that the resident will always have superior performance and that W will always exceed one. $W < 1$ would imply superior performance by the immigrant. The effects of reciprocal acclimation on fitness were measured at the following pairs of temperatures: 22°C and 32°C, 22°C and 37°C, 27°C and 37°C, 32°C and 37°C, and 37°C and 40°C.

Methods of serial transfer, temperature control, and com-

petition have been described in detail elsewhere (Bennett et al. 1992). Briefly, each genotype is stored as a clone that is frozen at −80°C. At the beginning of a set of competition experiments, each genotype was removed from the freezer and cultured for one day in Luria broth (LB) at 37°C and for a second day in Davis minimal medium (DM with 25 μg glucose ml^{-1}) at 37°C. On the third day—the acclimation day—each culture was diluted 100-fold into DM and 10 replicates of each genotype were placed at each of the two acclimation temperatures (40 cultures total). On the fourth day—the competition day—an aliquot of each of the two differently acclimated clones (possessing the reciprocal Ara markers) was diluted 200-fold into fresh DM; this mixed culture was immediately subsampled onto tetrazoleum arabinose (TA) indicator plates to determine R_i and I_i. Each flask was then incubated at one of the two competition temperatures for 24 h. At each competition temperature, 20 flasks were incubated, 10 having one Ara marker state as the resident and another 10 with the alternate marker state as resident. For example, in the 22°C and 32°C experiment, each incubator contained 20 competition flasks: 10 flasks containing Ara^- acclimated to 22°C and Ara^+ acclimated to 32°C and another 10 flasks containing Ara^- acclimated to 32°C and Ara^+ acclimated to 22°C. After 24 h, each culture was subsampled onto TA plates to determine R_f and I_f. In the 22°C and 32°C comparison, competition cultures were serially transferred in the competitive temperature environment for a second day to determine whether any fitness differential associated with acclimation in the initial competition persisted during the second day, by which time both the former immigrant, as well as the resident, should have been acclimated to the temperature of competition. In each experiment, the neutrality of the marker state was evaluated by a *t*-test. The beneficial acclimation hypothesis was evaluated by calculating 95% confidence limits about the mean fitness (using the *t*-distribution with df = 19): W significantly greater than 1 supports the hypothesis, and $W \leq 1$ does not.

Evolutionary Changes in Acclimation Effects

We next examined changes in the phenotypic acclimation response that occurred during evolution in new thermal environments. In our previous experiments (Bennett et al. 1992; Mongold et al. 1996), the two Ara genotypes served as the ancestors for experimental groups of replicate lineages placed in several different thermal environments. Here, we examined changes from the ancestral pattern of phenotypic acclimation responses in the groups that evolved at 32°C and 20°C. Previous experiments showed that both groups had undergone significant genetic adaptation to their respective selective environments, as judged by temperature-specific increments in their competitive fitness (Bennett et al. 1992; Mongold et al. 1996). To estimate changes in acclimation effects on fitness, we measured the mean fitness in the lines of each experimental group (either 32°C or 20°C) relative to the ancestor both at the selective temperature (32°C or 20°C) and at the ancestral temperature (37°C) after acclimation to either the selective or the ancestral temperature (experimental approach modified from Leroi et al. 1994b). The difference in fitness at the selective temperature when competitors were acclimated to this selective temperature (condition A) and when they were acclimated to the ancestral temperature (condition B) is used to estimate the evolutionary change in the effect of acclimation in the selective environment. The difference in fitness at the ancestral temperature when competitors were acclimated to this temperature (condition C) and when they are acclimated to their selective temperature (condition D) was used to estimate the evolutionary change in the effect of acclimation to the ancestral environment (see Fig. 2 for a diagrammatic design of these experiments). Note that *both* the evolved genotypes and their ancestors are acclimated to the same temperature prior to competition at either temperature. Relative fitness is now expressed as:

$$W = \log (E_f/E_i)/\log (A_f/A_i), \quad (2)$$

where subscripts i and f denote initial and final values for the population densities of the evolutionarily derived (E) and ancestral (A) genotypes.

Methods of serial transfer, temperature control, and competition were identical to those described in the previous section. The bacteria used in these experiments were the two ancestral genotypes (Ara^- and Ara^+) and single genotypes isolated from each of the six replicate lines in two evolutionary treatment groups: at 32°C, lines 32-1, 32-2, 32-3, 32+1, 32+2, 32+3, and at 20°C, 20-1, 20-2, 20-3, 20+1, 20+2, 20+3. The sign of these genotypes indicates Ara marker state; during competitive measurements of relative fitness, these genotypes were always paired with the reciprocally marked ancestor. For each selective temperature, the experiment was run in three blocks. At the beginning of an experimental block, each genotype was removed from the freezer and cultured for one day in LB at 37°C and for a second day in DM at 37°C. On the third day—the acclimation day—each culture was diluted 100-fold into DM and a replicate culture was placed at each of the two temperatures, the selective temperature (either 32°C or 20°C) and the ancestral temperature (37°C) (two ancestral and six derived genotypes at each of two temperatures = 16 cultures total). On the fourth day—the competition day—at each acclimation temperature, four replicate competition cultures were established by transferring an aliquot of each derived genotype and the ancestor (possessing the reciprocal Ara marker) into fresh DM (200-fold dilution of each). These cultures were immediately subsampled onto TA plates to determine E_i and A_i. Two of these four cultures were incubated for 24 h at the selective temperature and the other two, at the ancestral temperature. After 24 h, each culture was subsampled onto TA plates to determine E_f and A_f. In each experimental block, two replicates of each competition in each of two acclimation states were performed at both experimental temperatures (2 replicates × 6 derived genotypes × 2 acclimation states × 2 experimental temperatures = 48 cultures/block). The experiment was repeated three times, yielding six replicate measurements of fitness for each derived genotype in each acclimation state at each temperature. The mean value of fitness for each derived genotype was used to calculate the difference between experimental conditions (A − B) or (C − D), and the significance of the difference was evaluated by calculating 95% confidence limits about the mean difference (using the *t*-distribution with df = 5). Thus, our degrees of freedom for

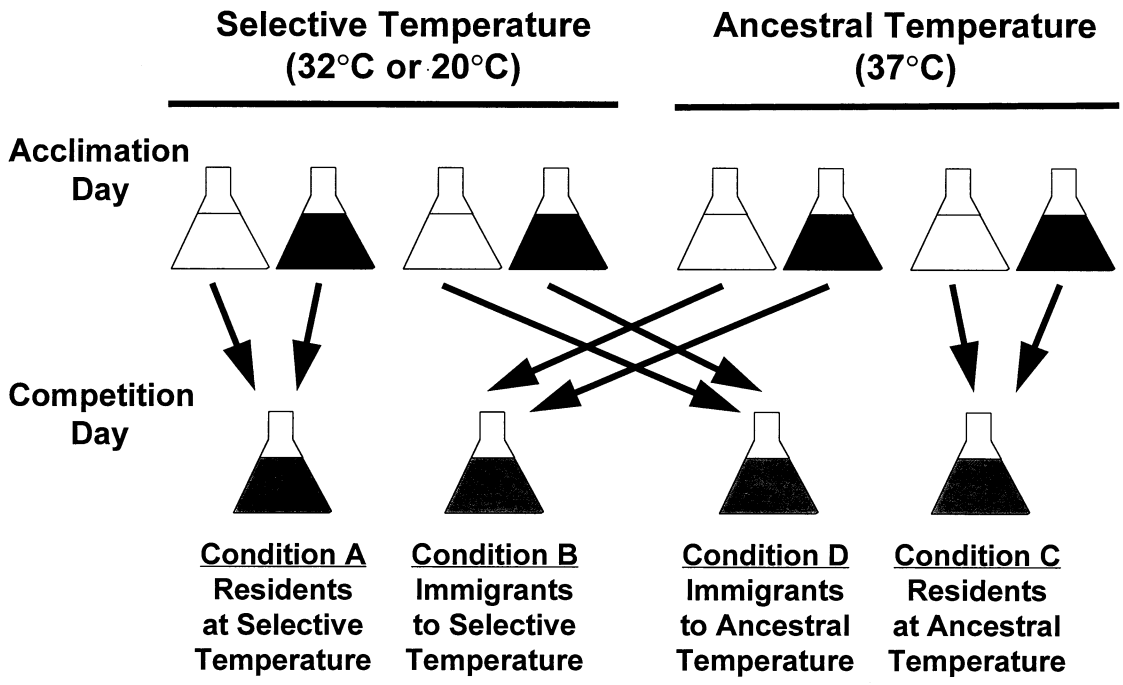

FIG. 2. Experimental design to investigate evolutionary changes in acclimation effects on fitness. The differences in shading indicate two genotypes that differ in their selective histories (as well as being genetically marked), one being the ancestral genotype and the other having evolved at the selective temperature (either 32°C or 20°C). Pairs of ancestral and evolved genotypes are grown separately at either the selective (32°C or 20°C) or ancestral (37°C) temperature on the acclimation day. They are then competed directly with each other either at their acclimation temperature (conditions A and C) or at the alternative temperature (conditions B and D). The difference in fitness at the selective temperature when competitors are acclimated to the selective temperature (condition A) and when they are acclimated to the ancestral temperature (condition B) measures the evolutionary change in the effect of acclimation to the selective environment. The difference in fitness at the ancestral temperature when competitors are acclimated to the ancestral temperature (condition C) and when they are acclimated to the selective temperature (condition D) measures the evolutionary change in the effect of acclimation to the ancestral environment.

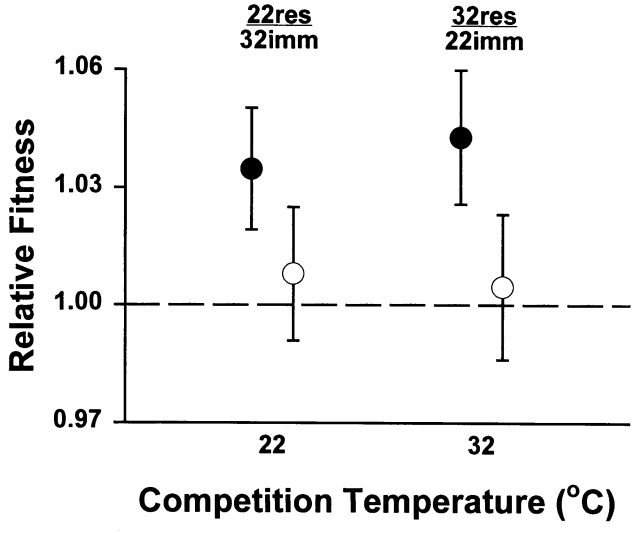

FIG. 3. Mean fitness (± 95% confidence limits) of the resident relative to the immigrant at acclimation and competition temperatures of 22°C and 32°C calculated over the first (solid symbols) and the second (open symbols) days of competition. During the first day, the resident is more fit than the immigrant at both competition temperatures, supporting the beneficial acclimation hypothesis in each case. During the second day, when both competitors have acclimated to the competition temperature, the fitness differential has disappeared, confirming the transitory, phenotypic nature of the acclimation response measured during the first day of competition.

testing evolutionary hypotheses were determined by the number of independently derived genotypes in each evolutionary treatment group, with all other aspects of the experimental design being balanced and fully blocked.

RESULTS

Fitness Effects of Phenotypic Acclimation in the Ancestor

Ara marker state was not a significant factor in any of the comparisons ($P > 0.1$) and was therefore excluded from subsequent analyses. Results of the 22°C and 32°C acclimation experiment are presented in Figure 3. During the first day of competition (Fig. 3, solid symbols), the residents had higher fitness than the immigrants at both competition temperatures ($P < 0.001$). These results conform to and support the beneficial acclimation hypothesis. The benefit is also apparently symmetrical, with no significant difference between the fitness advantage of the residents at the two competition temperatures ($P = 0.47$). During the second day of competition (Fig. 3, open symbols), these fitness differentials had disappeared: the relative fitnesses of the competitors at both temperatures were not significantly different from each other (that is, relative fitness is not significantly different from one) at either temperature. Thus, the beneficial effect of acclimation is a reversible phenotypic trait that disappears as the immigrant become acclimated to competition temperature.

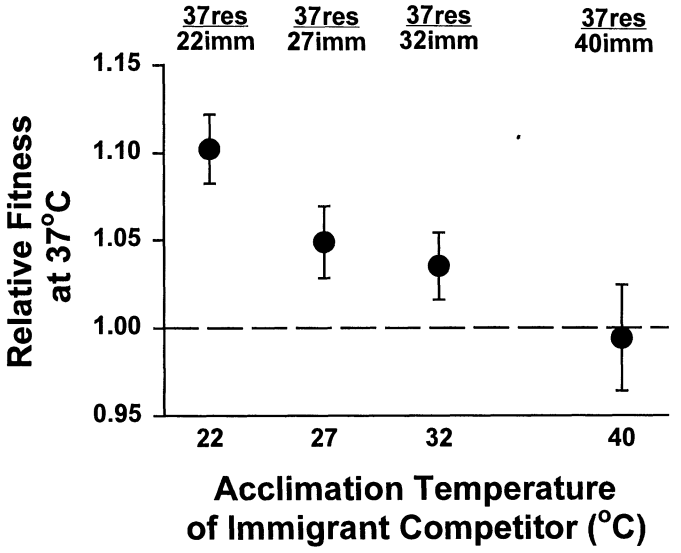

FIG. 4. Mean fitness (± 95% confidence limits) of the resident at 37°C relative to immigrants acclimated to different temperatures. The resident has a significant advantage relative to immigrants acclimated to 22°C, 27°C, or 32°C, in accord with the beneficial acclimation hypothesis. The resident has no significant advantage or disadvantage relative to an immigrant from 40°C.

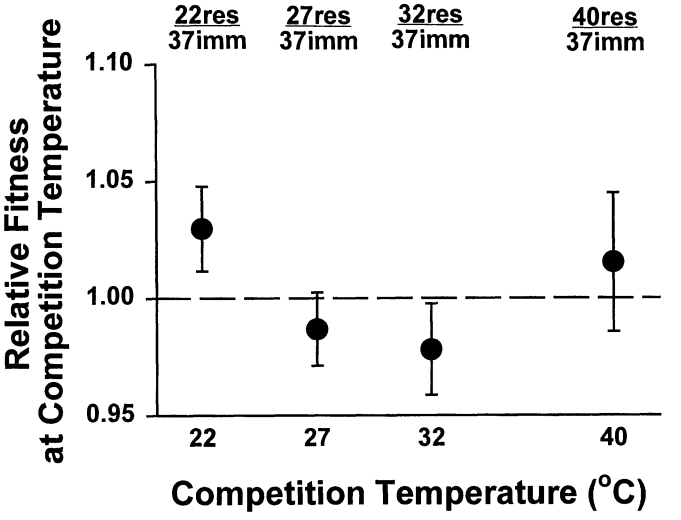

FIG. 5. Mean fitness (± 95% confidence limits) of the resident at various competition temperatures relative to an immigrant acclimated to 37°C. The resident has a significant advantage at 22°C, a result consistent with the beneficial acclimation hypothesis. At 32°C, however, the resident is at a significant disadvantage relative to the immigrant. At 27°C and 40°C, the resident has no significant advantage or disadvantage.

Fitness of the resident at 37°C relative to that of immigrants from several other temperatures is shown in Figure 4. The beneficial acclimation hypothesis is supported when the immigrants were acclimated to 22°C ($P < 0.001$), 27°C ($P < 0.001$), and 32°C ($P = 0.001$). That is, the 37°C-acclimated resident was more fit than the immigrant acclimated to these other temperatures. Further, over this range of immigrant acclimation temperatures, the benefit to the resident increases with the magnitude of the difference in acclimation temperatures ($F = 24.4$; df = 1,58; $P < 0.001$). However, neither resident nor immigrant from 40°C has any significant advantage ($P = 0.69$) in competition at 37°C.

Figure 5 summarizes the results of the reciprocal experiments in which an immigrant acclimated to 37°C competes at several temperatures against residents acclimated to those temperatures. The pattern of fitness advantage is very different from that found for the 37°C resident. At 22°C, the resident has a greater fitness than does the 37°C immigrant ($P = 0.003$), in accord with the beneficial acclimation hypothesis. However, at 27°C, mean fitness is 0.987, a value nearly significantly below one ($P = 0.09$), and at 32°C, mean fitness of 0.978 is significantly below one ($P = 0.03$). In the latter case and perhaps also in the former, the fitness of the immigrant from 37°C is actually greater than that of the resident acclimated to the competition temperature. This outcome is exactly the opposite of that predicted by the beneficial acclimation hypothesis and indicates that prior exposure to the competition environment is in fact detrimental when the immigrant comes from 37°C. At 40°C, again no fitness advantage or disadvantage accrues to acclimation at 37°C instead of 40°C ($P = 0.29$). Even for 22°C, where the beneficial acclimation hypothesis is supported for both competition temperatures, the benefit to the resident is not symmetrical, but is substantially greater in competition at 37°C that at 22°C ($P < 0.001$).

What might account for the divergent patterns evident in Figures 4 and 5, in which acclimation effects are clearly not reciprocal or symmetrical at different pairs of acclimation and competition temperatures? An important influence on these patterns might be the evolutionary thermal history of the bacterial lineage examined. Specifically, this genotype was isolated from a population propagated at 37°C for the preceding 2000 generations, during which time the population had undergone extensive adaptive evolution (Lenski et al. 1991). Certainly in the experiments reported above, acclimation to 37°C appears to entail advantages, sometimes even during competition at other temperatures. Perhaps acclimation to an organism's historical selective temperature generally confers such advantages, so that evolutionary adaptation to a particular temperature not only increases fitness at that temperature, but also extends to other thermal environments the beneficial effects of phenotypic acclimation to the selective environment. Fortunately, such hypotheses can be rigorously tested with our experimental evolutionary lines. In the next section, we examine the evolutionary modification of the ancestral acclimation patterns during 2000 generations of adaptation to two different thermal environments.

Evolutionary Changes in Acclimation Effect

The results of the fitness measurements with the 32°C-selected genotypes are summarized in Tables 1 and 2. The mean fitness estimates for both the direct and correlated responses of this group are in excellent agreement with those reported previously (condition A: 1.11, Condition C: 1.02; Bennett and Lenski 1996). During competition with the ancestor at 32°C (Table 1), prior acclimation to that temperature yields a significant advantage to the 32°C-selected genotypes:

TABLE 1. The effect of acclimation temperature on the fitness of six 32°C-selected lines during competition at 32°C. Each value is the mean of six replicate measurements of fitness of the selected line relative to the common ancestor. In condition A, both competitors (a genotype selected at 32°C and its ancestor) are separately acclimated to 32°C prior to competition. In condition B, both competitors are separately acclimated to 37°C prior to competition. We expect that fitness in condition A will exceed that in condition B if acclimation to 32°C confers an advantage over acclimation to the ancestral temperature of 37°C.

32°C-selected genotype	Condition A 32 → 32	Condition B 37 → 32	Difference (A − B)
32 − 1	1.088	1.046	0.041
32 − 2	1.162	1.118	0.045
32 − 3	1.104	1.051	0.052
32 + 1	1.135	1.017	0.118
32 + 2	1.130	1.069	0.061
32 + 3	1.179	1.102	0.077
Mean	1.133	1.067	0.066
		SE	0.012
		95% CI	0.036 to 0.096
		P	0.002

TABLE 2. The effect of acclimation temperature on the fitness of six 32°C-selected lines during competition at 37°C. Each value is the mean of six replicate measurements of fitness of the selected line relative to the common ancestor. In condition C, both competitors (a genotype selected at 32°C and its ancestor) are separately acclimated to 37°C prior to competition. In condition D, both competitors are separately acclimated to 32°C prior to competition. We expect that fitness in condition C will be less than that in condition D if acclimation to 32°C enhances fitness even during competition at 37°C.

32°C-selected genotype	Conditon C 37 → 37	Condition D 32 → 37	Difference (C − D)
32 − 1	0.996	1.063	−0.067
32 − 2	1.069	1.108	−0.039
32 − 3	1.012	1.070	−0.058
32 + 1	0.947	0.993	−0.046
32 + 2	1.061	1.114	−0.053
32 + 3	1.075	1.127	−0.052
Mean	1.027	1.079	−0.053
		SE	0.004
		95% CI	−0.043 to −0.062
		P	< 0.001

all six lines have higher fitness when both competitors are acclimated to 32°C instead of to 37°C (A − B > 0). This pattern is in distinct contrast to that seen in the ancestor (Fig. 5), in which acclimation to 32°C prior to competition at that temperature actually depressed fitness. When competition takes place at 37°C (Table 2), the 32°C-selected genotypes are significantly more fit if both competitors are acclimated to 32°C than if they are both acclimated to 37°C (C − D < 0). Again, this result is in marked contrast to the ancestral pattern of a benefit to acclimation to 37°C during competition at that temperature (Fig. 4). Clearly, evolutionary adaptation to 32°C has fundamentally altered, even reversed, the ancestral pattern of phenotypic acclimation effects. Whereas the ancestor benefited from acclimation at 37°C prior to competition at either 32°C or 37°C, the six 32°C-selected lines all benefit from acclimation to 32°C prior to competition at either 32°C or 37°C.

TABLE 3. The effect of acclimation temperature on the fitness of six 20°C-selected lines during competition at 20°C. Each value is the mean of six replicate measurements of fitness of the selected line relative to the common ancestor. In condition A, both competitors (a genotype selected at 20°C and its ancestor) are separately acclimated to 20°C prior to competition. In condition B, both competitors are separately acclimated to 37°C prior to competition. We expect that fitness in condition A will exceed that in condition B if acclimation to 20°C confers an advantage over acclimation to the ancestral temperature of 37°C.

20°C-selected genotype	Condition A 20 → 20	Condition B 37 → 20	Difference (A − B)
20 − 1	1.118	1.127	−0.009
20 − 2	1.096	0.989	0.106
20 − 3	1.140	1.028	0.112
20 + 1	1.103	1.050	0.053
20 + 2	1.098	1.194	−0.097
20 + 3	1.097	1.119	−0.022
Mean	1.109	1.085	0.024
		SE	0.033
		95% CI	−0.061 to 0.109
		P	0.51

Fitness measurements of the 20°C-selected genotypes are reported in Tables 3 and 4. Again, both the direct and correlated responses of this group are in very good agreement with those determined previously (condition A: 1.09, condition C: 0.97; Bennett and Lenski 1996). In competition with the ancestor at 20°C (Table 3), prior acclimation to 20°C has no significant effect on the average fitness advantage of the 20°C-selected genotypes (A − B ≅ 0). However, during competition at 37°C (Table 4), the fitness of the 20°C-selected genotypes is significantly enhanced by prior acclimation to 20°C, a pattern consistent across all six replicate lines (C − D < 0). This result contrasts to the ancestral pattern shown in Figure 4. Again, evolutionary adaptation, in this case to 20°C, has changed the ancestral pattern of phenotypic acclimation effects on fitness.

TABLE 4. The effect of acclimation temperature on the fitness of six 20°C-selected lines during competition at 37°C. Each value is the mean of six replicate measurements of fitness of the selected line relative to the common ancestor. In condition C, both competitors (a genotype selected at 20°C and its ancestor) are separately acclimated to 37°C prior to competition. In condition D, both competitors are separately acclimated to 20°C prior to competition. We expect that fitness in condition C will be less than that in condition D if acclimation to 20°C enhances fitness even during competition at 37°C.

20°C-selected genotype	Condition C 37 → 37	Condition D 20 → 37	Difference (C − D)
20 − 1	1.021	1.124	−0.102
20 − 2	0.953	1.076	−0.123
20 − 3	1.003	1.164	−0.161
20 + 1	1.022	1.115	−0.093
20 + 2	0.746	0.988	−0.242
20 + 3	0.971	1.066	−0.095
Mean	0.953	1.089	−0.136
		SE	0.024
		95% CI	−0.075 to −0.197
		P	0.002

Discussion

Acclimation in Bacteria and Other Organisms

The process of acclimation is usually studied in organisms with much longer generation times than those of bacteria, and exposure to a new thermal environment encompasses only a portion of an individual's life span (see studies in Prosser 1973; Hochachka and Somero 1984; Cossins and Bowler 1987). However, temperature exposure may be particularly important in influencing early phenotypic development (see Blaxter 1988; Atkinson 1996; Johnston et al. 1996), and the phenotype of an offspring may also be significantly influenced by parental acclimation state (Crill et al. 1996 and references therein). Some acclimation effects have even been reported to span three generations (Watson and Hoffmann 1995). Because of the potential for cross-generational effects, experimenters investigating acclimation have been cautioned to raise study organisms through at least two generations in a common-garden thermal environment (Crill et al. 1996). We therefore grew our bacteria for six to seven generations in the acclimation phase of these experiments to ensure that the organisms were fully conditioned to the intended environment prior to testing the competitive ability of the resulting phenotype. While multigenerational exposure to acclimation temperature is not usual in acclimation experiments, it in fact is a desirable feature in such studies.

The concern may arise whether this multigenerational acclimation period is so long that it may allow genetic (evolutionary) adaptation of the exposed populations to the acclimation temperature, in addition to strictly phenotypic changes. We do not believe that genetic adaptation occurred during these experiments for two reasons. First, the acclimation effects on fitness were shown to be completely eliminated during further exposure to the competition temperature (contrast solid and open symbols in Fig. 3). In contrast, genetic changes arising from selection on new mutations within the competing populations should have led to even greater fitness differentials on the second day of competition. Thus, the observed disappearance of the fitness advantage is consistent with its phenotypic nature. Second, we can calculate the magnitude of the fitness advantage that would be required for a hypothetical new mutation to yield a population mean fitness of 1.03 (Fig. 3) after only 13–14 generations (acclimation and competition days). Assuming a beneficial mutation in one cell at the beginning of the acclimation period, and given the known population sizes of the two competitors, the relative fitness of the hypothetical mutant would have to be approximately 2.3 to increase mean fitness by 3% in this short time period. Such a mutant would very rapidly sweep through the population and establish a population mean fitness of 2.3 well within the first 100 generations. However, in evolution experiments involving selection of the same ancestral populations at these temperatures, we never observed a fitness change of this magnitude: fitnesses after 2000 generations at 20 and 32°C increased to only approximately 1.1 (Bennett et al. 1992; Mongold et al. 1996). It is virtually impossible that mutations of such large beneficial effect would have occurred in all of the acclimation experiments, but never in the evolution experiments. On of both these grounds, we therefore conclude that the rapid and reversible changes that occurred during the acclimation treatments in this study are, in fact, due to phenotypic acclimation and not genetic adaptation. Thus, these experiments provide an appropriate model for analyzing the effects of phenotypic acclimation on performance.

Generality of the Beneficial Acclimation Hypothesis

Does acclimation to a temperature confer an advantage in competition at that temperature, as predicted by the beneficial acclimation hypothesis? According to the results of experiments with the ancestral bacterium, the answer is "sometimes." The 22°C and 32°C cross-competition experiments (Fig. 3, solid symbols) provide a classic example of reciprocal and even symmetrical acclimation benefits. Another example is the pattern of increasing benefit to the 37°C-acclimated resident in competition with immigrants acclimated to progressively lower temperatures (Fig. 4). However, acclimation accrues no benefit in cross-competition experiments between 37°C and 40°C (Figs. 4 and 5), and it is even detrimental when a 32°C-acclimated resident (and perhaps a 27°C-acclimated resident) competes with a 37°C-acclimated immigrant (Fig. 5). Thus, a significant benefit to acclimation at the competition temperature was found in only a bare majority of the cases investigated (7 of 12 comparisons, including the two from Leroi et al. 1994a). Evidently, acclimation may sometimes be beneficial, but it is hardly necessarily so. The general hypothesis of an invariable benefit to acclimation has to be rejected.

The finding that a 37°C-acclimated immigrant is more fit than a 32°C-acclimated resident (Fig. 5) is of particular interest for several reasons. First, it indicates that the fitness reduction associated with acclimation that we previously reported (for a 32°C-acclimated immigrant and a 41.5°C-acclimated resident, Leroi et al. 1994a) is not a unique case. Second, this decrement cannot be attributed to an effect of stress. Although 41.5°C is stressful by several measures to this genotype of *E. coli*, 32°C is not (Lenski and Bennett 1993). Hence, the decrement in competitive fitness of a resident at 32°C relative to an immigrant from 37°C cannot be an effect of the production and action of stress proteins, as we suggested might account for the resident's handicap at 41.5°C (Leroi et al. 1994a). We do not know the mechanisms underlying the advantages and disadvantages of acclimation observed in the current experiments. It is clear, however, that the paradoxical harmful effect of acclimation to the competition temperature may arise even in benign thermal environments. Third, on the basis of the results of Leroi et al. (1994a), one might have suggested that acclimation to 32°C confers some inherent competitive superiority in this genotype, a prediction consonant with the optimal acclimation temperature hypothesis (Huey and Berrigan 1996). However, the results of this study indicate that the 32°C-acclimated form wins in some circumstances and loses in others. If anything, the results of the current study suggest that acclimation to 37°C, the ancestral temperature of the genotype, confers some inherent superiority, even for an immigrant to other competition temperatures. A similar situation, in which benefit at other temperatures accrues to acclimation at the an-

cestral temperature, has been previously noted by Zwaan et al. (1992) and Zamudio et al. (1995) in *Drosophila*. It was this concordance between beneficial acclimation effects and selective history that prompted us to test the association further using genotypes adapted to other thermal environments.

Evolutionary Changes in Acclimation Effects

The post hoc interpretation of the experiments outlined above suggested that acclimation benefit may be conditioned by evolutionary history. Specifically, the genotype that was studied had been isolated from a population that was grown at 37°C for 2000 generations and had undergone extensive genetic adaptation to that temperature (Lenski et al. 1991). When acclimated to 37°C, this genotype was more fit in competition at that temperature than most immigrant acclimation states (Fig. 4). This result is not particularly surprising, being predicted by the beneficial acclimation hypothesis. However, acclimation to 37°C also benefited this genotype when it was an immigrant to certain other temperatures (Fig. 5), resulting in a pronounced asymmetry of fitness effects associated with acclimation to 37°C. This pattern may have two alternative explanations. First, perhaps this is a unique environment for *E. coli* because acclimation to 37°C invariably benefits this species for some (unknown) physiological reason. Second, perhaps beneficial effects of acclimation accrue to whatever has been the organism's historical selective temperature. In this latter case, 37°C is special only because of the particular selective history of this genotype. This explanation implies that beneficial effects of acclimation are more prevalent when there has been a history of selection in the corresponding thermal environment. It further implies that genetic adaptation to a certain thermal environment may extend the benefits of acclimation in that environment even to competition in other environments.

We were able to test these alternative explanations by examining changes in the fitness effects of acclimation in derived genotypes that had evolved under, and adapted to, different thermal regimes. The first explanation (superiority at 37°C) predicts that the effects of acclimation on fitness should be unaltered in these derived genotypes, so that 37°C-acclimated bacteria should retain their advantage relative to those acclimated to other temperatures. The second explanation (superiority at historical temperature) predicts that the beneficial effects of acclimation should follow the temperature of genetic adaptation; specifically, those genotypes adapted to 32°C or 20°C should no longer be disadvantaged when acclimated to their selective temperatures relative to 37°C-acclimated forms.

Observations on genotypes adapted to 32°C and 20°C (Tables 1–4) strongly support the latter explanation: evolutionary history alters the pattern of phenotypic acclimation effects so that benefit tends to accrue to acclimation to the new selective temperature. In the 32°C-adapted genotypes, the ancestral pattern is completely reversed: bacteria acclimated to 32°C have higher fitness in competition at both 32°C and 37°C (Tables 1 and 2), instead of the ancestral pattern of lower fitness at both temperatures (Figs. 4 and 5). Even in the 20°C-adapted group, acclimation to the historical temperature of 20°C confers a benefit during competition at 37°C (Table 4), a particularly surprising result considering the 17°C difference in acclimation and competition temperatures. It is important to keep in mind that these acclimation benefits at 37°C are only correlated and not directly selected responses: the experimental populations from which these genotypes were taken had not experienced 37°C during the most recent 2000 generations of their selective history.

The foregoing results demonstrate that evolutionary adaptation may alter or even reverse ancestral patterns of phenotypic acclimation. Unexpected changes in acclimation effects may occur even when acclimation ability is potentially subject to direct selection: we previously showed that acclimation benefit paradoxically declined during evolution in a variable environment that was characterized by abrupt thermal transitions (Leroi et al. 1994b). Consequently, no comprehensive generalizations emerge about the relationships between phenotypic acclimation and genetic adaptation. It is clear, however, that the latter frequently alters the former and often favors acclimation to the historical selective environment, even when competition occurs in some other environment. For each of the three historical selective temperatures examined (20°C, 32°C, and 37°C), we found at least one alternative thermal environment in which an immigrant acclimated to the historical temperature is competitively superior to a resident acclimated to that alternative environment.

Conclusions

Our experimental study system has two distinct advantages for evaluating hypotheses about phenotypic acclimation and its evolution. First, the system permits direct measurement of fitness, as opposed to evaluation of presumptive fitness components. Second, the system allows the construction and analysis of lineages of organisms that have evolved in, and genetically adapted to, controlled and defined environments. Results from this experimental system suggest that phenotypic acclimation may sometimes, as expected, be beneficial in promoting fitness. However, frequently no such benefit occurs, and sometimes it is actually disadvantageous to acclimate to an environment prior to competition in that environment. It is also clear that patterns of acclimation effects on fitness are easily influenced by genetic adaptation to historical selective environments. Acclimation to those historical temperatures apparently accrues benefit not only during competition at those temperatures, but also during competition over a wide range of other temperatures.

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